
ONION SMUDGE

BY

J. C. WALKER UNIVERSITY OF WISCONSIN

PH. D. THESIS 1918

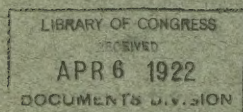
Reprinted from JOURNAL OF AGRICULTURAL RESEARCH

Vol. XX, No. 9 : : : Washington, D. C., February 1, 1921



PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH
THE COOPERATION OF THE ASSOCIATION OF LAND-GRANT COLLEGES

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1921



SB608
05W3

6.5 B. my. 26.22.

ONION SMUDGE

By J. C. WALKER

Assistant Professor of Plant Pathology, University of Wisconsin, and Pathologist, Office of Cotton, Truck, and Forage Crop Disease Investigations, Bureau of Plant Industry, United States Department of Agriculture¹

INTRODUCTION

Smudge is a common disease of onions occurring both in the field and in storage or transit. It is confined for the most part to the bulbs and is characterized by dark green to black spots of variable size and shape on the outer scales. The spots may be homogeneous in appearance or may consist of numerous individual stromata scattered miscellaneously or arranged in concentric rings. The disease is most common on the white varieties of onions and damages materially the appearance and market value of the crop. The causal fungus has heretofore generally been known as *Vermicularia circinans* Berkeley, but as explained later in this paper it should more properly be termed *Colletotrichum circinans* (Berk.) Voglino.

The present investigations have been carried on with special reference to the disease as it occurs in the districts of southeastern Wisconsin and northeastern Illinois where onion sets are grown. The growing of white onion "bottom sets" is an industry of considerable importance in these sections, and the methods used in growing and handling the set crop are often conducive to the excessive development of smudge during and immediately following harvest. In this study attention has been given primarily to the mycological and physiological aspects of the causal organism, the relation of the parasite to the host tissue, the life history of the fungus with relation to the production of disease, and the development of remedial measures.

THE DISEASE

COMMON NAMES

A number of common names have been used in American and European literature for this disease—namely, "onion *Vermicularia*" (3)², "*Vermiculariose*" (29), "black spot" (7, 30), "scab" (17, 21), "anthracnose" (7, 36, 37, 38), and "smudge" (26). The name "anthracnose"

¹ This study was begun in the Department of Plant Pathology at the University of Wisconsin in 1914, and the major portion was completed in 1917. Since the writer entered the Office of Cotton, Truck, and Forage Crop Disease Investigations in the latter year, observations have been extended to sections outside of Wisconsin. Grateful acknowledgments are expressed to Dr. L. R. Jones, under whose immediate direction the work has been done, and to Drs. J. J. Davis and E. M. Gilbert, who have given valuable aid and suggestions on the mycological phases of the problem.

² Reference is made by number (*italic*) to "Literature cited," p. 719-721.

has been much used up to the present time. However, since the symptoms have little in common with those of the more common anthracnoses, and since it is believed that as simple and as descriptive a name as possible should be chosen, the name "onion smudge" is used in this paper to designate the disease, and this name is recommended for general usage.

HOST PLANTS

White varieties of the onion (*Allium cepa*) are the chief ones affected by smudge, but all varieties thoroughly tested have been found susceptible to at least a slight degree. The disease also occurs on shallots (*A. ascalonicum*) and on leek (*A. porrum*). It has never been found on garlic (*A. sativum*).

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Onion smudge was first described in 1851 by Berkeley (4) in England, where it was found on the outer scales of a white variety. Subsequent reports of its occurrence in Europe have been made by Massee (17) in England, Bubák (8) in Bohemia, and Voglino (35) and Allescher (1) in Italy.

The first collection of this disease in America, made by Michener, was reported by Berkeley (5) in 1874. Since that time it has been recorded in literature as occurring in Rhode Island (3), Connecticut (10, 19, 33), New York (20, 22), New Jersey (13, 25), Ohio (26), Indiana (21, 34), Illinois (30), Wisconsin (23), and Alabama (2). Additional data furnished by the Plant Disease Survey show that it has been present also in Massachusetts, Pennsylvania, Delaware, Maryland, Virginia, Georgia, Louisiana, Texas, Minnesota, and Iowa.

It is thus a disease of widespread occurrence; and, indeed, when one considers the fact that thousands of bushels of infected "bottom" sets are being shipped annually to all parts of the country and abroad, it is reasonable to suppose that its distribution is even more general than this summary indicates.

DESCRIPTION OF SMUDGE (PL. 80, 81)

The disease is confined entirely to the scales and the lower portions of the unthickened leaves which constitute the neck of the bulb. It first becomes manifest upon the appearance of minute stromata which form just beneath the cuticle of the host. These are dark green at first, becoming black with age. Depending on conditions of infection, the individual stromata may be scattered miscellaneously over the surface of the bulb, or, as is more commonly the case, they may be congregated in smudgy spots around a few centers of infection. These spots are usually roughly circular and variable in size. They often coalesce and occasionally contain stromata arranged in concentric rings. Under moist conditions the stromata bear acervuli which contain prominent setae readily distinguished with a lens of low magnification. Cream-colored spore masses frequently form on these fruiting bodies.

Penetration of underlying dry scales by the fungus causes similar spots, which are commonly surrounded by yellowish borders. On the fleshy scales the disease first appears as minute, sunken, yellowish spots which gradually enlarge and often coalesce. As the disease progresses, the black stroma of the fungus usually appears; and, with the collapse of the host cells, spots very similar to those on the dry outer scales result. When the dark-colored stroma does not develop before the scale has entirely dried down, the affected portions appear as slightly raised, yellowish spots, giving to white onion sets an unnatural color which is almost as detrimental to their market value as the black, smudgy spots.

The disease makes its appearance early in July under Wisconsin conditions, the fungus living on the outer dead scales and increasing in amount up to harvest time, when the outer two or three scales may be affected. From this time on it penetrates farther into the bulbs, progress depending upon environmental conditions. Badly diseased bulbs tend to sprout prematurely in storage. In most severe cases the fungus penetrates the entire bulb and causes a complete collapse of the fleshy scales.

The foregoing description applies to the disease as it appears on white onions. On colored varieties (red, yellow, and brown) the fungus is confined, with rare exceptions, to the neck of the bulbs where there is little or no pigment in the tissue, and the symptoms in these cases resemble closely those on the corresponding parts of the white varieties.

On shallots the disease appears as smudgy spots very similar to those on onion and is confined to the outer leaves or scales. On leeks similar symptoms prevail.

OTHER DISEASES LIKELY TO BE CONFUSED WITH SMUDGE

Onion bulbs as they mature are subject to attack by a number of fungi which develop saprophytically on the dead outer scales and produce symptoms which may easily be confused with those of smudge. The most common of these are two species of *Macrosporium* (*Macrosporium porri* Ell. and *M. parasiticum* Thüm.) (33), and a species of *Phoma*, probably *Phoma alliiicola* Sacc. and Roum. (24). The *Macrosporiums* produce irregular, dark green spots which are due to ramification of the mycelium through the dead scales, but which lack the stromata and more or less regular outline of the smudge spot. In a moist atmosphere the fungi fruit and develop a dark green mold due to the production of conidia (Pl. 81, F, G). In rare instances black perithecia of *M. parasiticum* are found on the outer bulb scales. *Phoma* produces small black pycnidia which are often difficult to distinguish macroscopically from the stromata of the smudge fungus. It is commonly associated with *M. porri* (Pl. 81, H). These two fungi commonly attack both white and colored varieties, and in the latter case the pigment in the outer scales is usually destroyed, giving a symptom which is known in the trade as "onion blotch."

Onion smut is sometimes confused with smudge, especially when the former occurs on mature bulbs. In such instances, however, smut usually causes slightly raised, linear lesions which on colored varieties are commonly accompanied by more or less destruction of pigment. The exposure of the powdery spore mass upon breaking of the lesion establishes the identity of the smut fungus.

ECONOMIC IMPORTANCE

The importance of smudge as a detriment to the onion crop may properly be considered from three standpoints—(1) that of reduction of market value as a result of marred appearance, (2) that of actual shrinkage of the bulbs in storage, due to fungus invasion, and (3) that of increased sprouting of onion sets during storage. Thaxter (33) calls attention to the reduction of market value caused by smudge, citing an estimate by one grower of an actual loss of several thousand dollars to his crop in one season on this account. There is little doubt that marked spotting by this disease hampers greatly the disposal of white onions, since they are usually grown at a greater expense than colored varieties for a fancy trade which is prone to discriminate against disfigured stock. Under prolonged storage smudge causes a distinct shrinkage of the bulbs and promotes premature sprouting. These last two factors are not usually of material importance on large bulbs, but they are of much significance with respect to onion sets. The latter are usually harvested in August and September and kept in storage until March. The small bulbs are thus subjected to fungus invasion for several months, and data presented later in this paper show that in badly diseased sets the shrinkage may be doubled by smudge during this period.

Sets which sprout badly during storage are a total loss to the owner, since they will not stand shipping and must be discarded. Much of the sprouting of white sets in storage is due to severe attacks by smudge. Experimental data in support of this statement are given later in this paper.

It will be seen, therefore, that smudge is of greater importance than would be suspected from casual observation. In the Chicago district alone, where approximately 1,000,000 bushels of sets are grown annually, the aggregate loss due to shrinkage in weight and sprouting probably runs into many thousands of dollars.

CAUSAL ORGANISM

MORPHOLOGY

The morphology of the causal organism has previously been discussed by Berkeley (4), Thaxter (33), Stoneman (32), Stevens and True (30), and Kempton (16).

MYCELIUM.—The mycelium ranges from 2 to 8 microns in width, is septate and branching, varying widely with age as to color and size. It

is at first hyaline with few septa, but later the walls thicken and take on a dark green color, oil droplets become more numerous, and septation is more frequent.

STROMATA.—By close intertwining of the thick-walled mycelial threads, dark green to black stromata, usually only a fraction of a millimeter in diameter and few to several hundred microns thick, are formed beneath the cuticle of the host (fig. 1). On nutrient media these stromata commonly coalesce, forming a black stromateoid layer at the surface of the substrate. This coalescence sometimes occurs on the host, but more often the stromata remain distinct and are connected with one another by threads of the dark-colored mycelium. During protracted storage, or under poorly ventilated conditions, excessive stromatal development may occur (Plate 83, B). Thaxter (33) describes large, somewhat flattened sclerotia, "jet black externally and white within,"

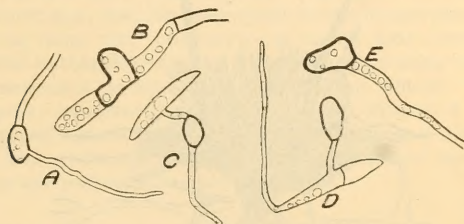


FIG. 1.—Conidia and appressoria of *Colletotrichum circinans*. The fusoid conidia (C, D) germinate by one or more germ tubes, often becoming septate during the process (D). Dark-colored, thick-walled appressoria develop at the tip of the germ tubes, usually as the latter come in contact with the host cuticle (C, D). Subsequent germination of appressoria commonly occurs (A, C). Terminal or intercalary appressoria-like cells, or chlamydospores, commonly develop within infected scales (B, E). Camera-lucida sketch. $\times 750$.

associated with the disease, though he does not definitely state that they are connected with the causal organism. The writer has never found bodies of this sort connected with the disease. On the other hand, sclerotia of *Botrytis* spp., which cause decay of onion bulbs and are commonly associated with smudge, compare favorably with his description.

APPRESSORIA OR CHLAMYDOSPORES.—(Fig. 1). These bodies are variable in size, dark brown in color, thick-walled, egg-shaped or roughly circular, usually terminal but occasionally intercalary. In germination drops on glass slides they form most abundantly where the germ tube comes in contact with the slide and less commonly in the upper region of the drop. Under such conditions they measure 6.5 to 8 microns by 4 to 5.5 microns. In Petri-dish cultures on various types of nutrient agar they are almost invariably produced at the tips of hyphae which come into contact with the glass surface. When "infection drops" containing

viable conidia are placed on the surface of onion bulbs, appressoria or chlamydo-spores are formed in contact with the scale. Later they send out germ tubes which penetrate the host. They are also commonly found within the tissue of affected scales.

ACERVULI.—The fruiting bodies are formed on the stromata which develop beneath the cuticle of the host. Short, hyaline conidiophores form in a palisade layer and rupture the cuticle of the host (fig. 2). One to several acervuli form on a single stroma. In the study of the morphology of the fruiting body the writer has found no evidence of a closed or partially closed receptacle, as described originally by Berkeley (4). Its true nature is more nearly in accord with the work of Stoneman (32), who found not a pycnidium but an open fruiting body.

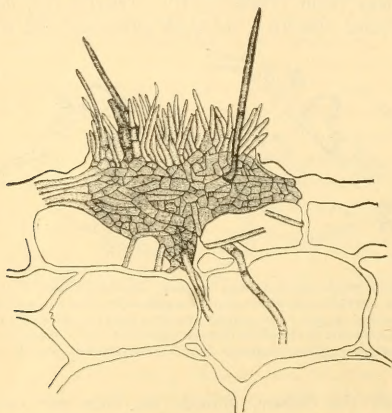


FIG. 2.—Acervulus of *Colletotrichum circinans* on artificially inoculated onion scale. Note the development of the stroma in the subcuticular wall and the rupture of the cuticle by the formation of the palisade layer of the sporiferous hyphae. Camera-lucida outline. $\times 265$.

SETAE.—Scattered throughout the acervulus are numerous setae arising from the basal stroma. They are thick-walled, dark-colored, 0 to 3 septate, upwardly attenuate, and 80 to 315 microns in length.

CONIDIA.—The conidia are borne acrogenously, being budded off one at a time. They are fusiform, continuous, hyaline to slightly ochraceous, somewhat curved, and obtuse at the very apex. Typically one prominent vacuole is present in the center of the conidium, but under some conditions the cytoplasm may contain many large vacuoles. As the spores are budded off from the conidiophores they form a cream-colored, somewhat mucilaginous mass on the top of the fruiting body. The spores vary from 14 to 30 microns in length and from 3 to 6 microns in width. A large majority, however, fall within the limits of 18 to 28 microns by 3 to 4 microns. They germinate usually by one, but occasionally by two or

three germ tubes, which are pushed out at any point on the surface. Septation of the spore commonly occurs during germination.

PERITHECIA.—Stevens and True (30) report the development of an ascigerous form on onion sets heavily infected with *Colletotrichum circinans* and have referred the same to the new genus *Cleistothecopsis*. The writer has never been able to prove *C. circinans* to be connected with any ascigerous form found on onion. Stevens and True claim the connection between the perithecia of *Cleistothecopsis* and *C. (Volutella) circinans* on the following evidence:

(1) they occurred on sets badly infected with the *Volutella*; (2) no other fungi or other types of mycelium were seen to be connected with them; (3) when studied in various stages of development, the typical *Volutella* mycelium, which offers definite characters for recognition, was seen in organic connection with them, as illustrated in figure 18 (1), (4) the outgrowths from the perithecia are like those of the *Volutella*.

This evidence is hardly sufficient to prove that the two forms are stages of the same fungus, especially since a large number of saprophytic or semi-saprophytic forms very commonly occur on the dead outer scales of onion bulbs and the differentiation of these from *C. circinans* on the basis of the characters of the mycelium is sometimes very difficult. The writer has, therefore, considered it advisable to use the binomial of the imperfect form until cultures from a single ascus or ascospore of the ascigerous form are shown to be identical with *C. circinans* both as to morphological characters and pathogenicity upon onion bulbs.

TAXONOMY

The taxonomic questions involved in this study concern first, the proper position of the fungus in the present system of classification, and second, the possible identity of the organism with other described species.

Berkeley (4) in the original description of the fungus refers to the fruiting body as a perithecium and places it in the genus *Vermicularia*, giving it the name *Vermicularia circinans*. Thaxter's (33) description implies that the fungus has an open fruiting body, but he states that in the early stages of its development a "sort of membrane" extends over the basidia. Miss Stoneman (32) describes a thick basal stroma bearing an open fruiting body. She also suggests that the characters of the fungus resemble more closely those of the genera *Colletotrichum* and *Volutella* than of *Vermicularia*. Voglino (35), believing the fruiting body to be an acervulus, which would thus place the organism in the order Melanconiales, transferred the species to the genus *Colletotrichum*. However, he gives no report of any study of the formation of the fruiting body.

Stevens and True (30) in discussing the fungus describe a sporodochium consisting—

of a pseudoparenchymatous inner tissue covered by a continuous surface layer... The young sporodochium eventually ruptures its covering membrane... In all cases the conidiophores are borne upon a raised superficial base which constitutes the sporodo-

chium, in contradistinction to the innate form of the acervulus which has no such base. The tubercular swelling, due to the massing of mycelium below and in the epidermis, partakes of sporodochial character also, and while this subepidermal part may not be regarded as constituting a true sporodochium it serves to emphasize the tendency of the fungus to produce such structures. . . The structure is a tubercle with a differentiated cortical outer layer. This outer layer ruptures and the tubercle develops as a sporodochium. . . These facts exclude the fungus from *Vermicularia* and place it in the *Tuberculariaceae* under *Volutella*.

In the discussion later in this paper on the relation of the parasite to the host it is shown that the development of the fungus commonly begins in the outer wall of the epidermal layer of host cells. As the cellulose becomes softened the hyphae multiply and a definite stroma forms within this softened cell wall. Mycelium penetrates the epidermal and underlying cells, and if humid conditions prevail the stroma will soon occupy several layers of subepidermal cells. In good storage this process is comparatively slow, but during a protracted period, especially if the humidity rises considerably from time to time, the stroma commonly does acquire a thickness of several hundred microns. An examination of many sections has shown that regardless of the extent of its development the stroma is always covered by the cuticle of the host. At the instant of sporulation a palisade layer of hyaline hyphae interspersed with dark-colored setae arises from the stroma, and in this process the cuticle is ruptured. This is shown to occur on stromata of widely different ages in figure 1 and Plate 83, B. It is to be noted in the first illustration that the stroma is of recent development, that it is confined to the outer wall of the epidermal layer, and that the cuticle has been ruptured only by the formation of the acervulus. In the second illustration, although the stroma is much greater in extent, the host cuticle is still to be found intact except where it has been ruptured by the two acervuli.

As pointed out by Saccardo (24, v. 3, p. 221-222, 233), certain species of *Vermicularia* are characterized by imperfect or cup-shaped pycnidia, and such forms approach the genus *Colletotrichum*. Obviously it is often difficult to determine the exact nature of the fruiting bodies, and as a result many forms belonging in *Colletotrichum* have been placed in *Vermicularia*. In the form under consideration there is no suggestion of pycnidial development at any time during the development of the fruiting body. On the other hand, it does fall within the limits of the genus *Colletotrichum*. It is true that the basal stroma is much more highly developed than in many of the better-known species of this genus. However, well-developed stromata have been described in several species of this genus, including *Colletotrichum antirrhini* by Stewart (31) and *C. cereale* by Selby and Manns (27). In both cases the stroma develops beneath the cuticle, which is ruptured only upon the formation of the acervulus.

It is quite possible that a critical study of the closely related species classified at present in *Vermicularia* and *Colletotrichum* will lead to the separation into another genus of those forms which develop acervuli above

thick basal stromata. This question, however, is not within the province of the present paper. Those species of the Hyphales which are placed in the family Tuberculariaceae are characterized by the grouping together of the sporiferous hyphae in a superficial, conglomerate, sessile, or stipitate mass, known as a sporodochium (24, v. 4, p. 635, 682). As already pointed out, Stevens and True (30) considered the fruiting body of the onion smudge organism to be of this nature and on that basis have transferred it to *Volutella*. In their description and figures, however, they seem to have interpreted the host cuticle as part of the so-called tubercle and thus as being of fungus origin. Were this true, the stroma would be superficial, and the fungus would properly belong to the genus *Volutella*. However, since the stroma is always subcuticular and the sporiferous hyphae are subcuticular in origin, the form is more characteristic of *Colletotrichum* than of *Volutella*. Here again it is obvious that these two genera need more critical study before their limits can be satisfactorily defined. Meanwhile in the light of evidence just given, the writer considers it more suitable to use the name *Colletotrichum circinans* (Berk.) Voglino for the onion smudge organism.

The comparison of *Colletotrichum circinans* with other related species has been very limited in this investigation. The list of species of this genus which coincide closely with the one in question as to spore measurements and general characters is large and extends over a wide host range. Obviously the comparison of herbarium specimens is insufficient basis for final conclusions under the circumstances. Critical comparison has been confined to *C. fructus* (S. and H.) Sacc., described as causing a fruit rot of apple. This species was originally described as a species of *Volutella* (28), but it was later transferred to *Colletotrichum* by Saccardo (24, v. 13, p. 1201)—

on account of the black setae and the acervulus being originally subcuticular.

Cross sections of apple fruits affected with *C. fructus* and with *C. circinans* are compared in Plate 83, C, D. In both cases the development of the stroma beneath the cuticle, which is ruptured only upon the formation of the acervuli, is clearly shown. The former species was chosen for comparative study because the spore measurements and general characters as previously described were closely similar to those of the onion smudge organism and authentic cultures were available.

Cultures of the apple organism or diseased fruits were secured from Prof. C. R. Orton, State College, Pa., Dr. L. R. Hesler, Ithaca, N. Y., Dr. Charles Brooks, Washington, D. C., and Mr. G. A. Meckstroth, Columbus, Ohio. Cross inoculation on apple and onion showed that *Colletotrichum circinans* was able to produce a rot of apple fruit similar to that produced by *C. fructus* (see Pl. 84, C). The formation of stromata and acervuli by both species on apple is shown in Plate 83, C, D. The rate at which the rot progressed, however, was uniformly slower in *C. circinans*. On onion,

C. fructus developed on the dead outer scale of the bulb, but no evidence of further invasion as occurs with *C. circinans* was observed. Thus, the two species are distinct as to pathogenicity.

Measurement of many hundreds of spores of several strains of both species produced on several substrates including the natural ones—namely, apple and onion—showed that the variations due to differences between strains and substrates along with differences due possibly to slight changes in environmental conditions precluded any distinction on this basis. The slight difference in the shape of spores shown in figure 3 was quite uniform. The spores of *Colletotrichum fructus* have walls nearly parallel throughout the middle half, and one end narrows much more abruptly than the other.

A comparison of growth on potato agar gave further evidence as to the distinction of the two species. The chief points of difference in development on this medium are as follows: (1) *Colletotrichum fructus*

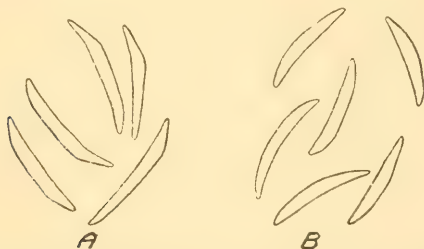


FIG. 3.—Spores of *Colletotrichum fructus* (A) and *C. circinans* (B). Note the slight difference in shape. In longitudinal section the walls of *C. fructus* are the more nearly parallel throughout the middle half, while at one end they converge more abruptly. Camera-lucida sketch. $\times 750$.

grows the more rapidly, (2) appressoria at the tips of hyphae coming in contact with the glass surface in plate cultures are absent in *C. fructus*, (3) the method of branching is quite distinct—that of *C. circinans* is dichotomous while that of *C. fructus* tends to be monopodial in that nearly straight threads of mycelium, which become dark-colored very early and are greater in diameter, run out radially from the center of the colony and send out hyaline side branches of less diameter. Stromata develop at various points from these radial hyphae. This mode of growth gives a somewhat stellate macroscopic appearance to the colony, which differs from that of *C. circinans*, where distinctly radial hyphae are absent and stromata are scattered. This macroscopic difference is shown in Plate 84.

Thus, although the morphological characters are only slightly variant, the two forms are considered distinct (1) because of difference in pathogenicity, (2) because of difference in spore shape, and (3) because of difference in type of colony on potato agar.

PHYSIOLOGY

ISOLATION OF THE FUNGUS

Pure cultures of the causal organism are readily obtained by the ordinary spore-dilution method. On potato-dextrose agar colonies appear in three to five days. Single spore strains were isolated from such cultures by means of the method described by Keitt (15). Isolations thus made from many lots of diseased material collected in Wisconsin, Illinois, Ohio, Connecticut, and Louisiana have yielded strains which are closely similar in their behavior.

CULTURAL CHARACTERS

ON POTATO AGAR (2 PER CENT DEXTROSE) PLATES.—(See Pl. 84, D, E.) The conidium germinates within 6 to 8 hours, sending out one to three hyaline germ tubes, which within 24 hours are many times the length of the spore. Colonies become macroscopic in about 2 days. The mycelium becomes somewhat thicker and denser in the center of the colony, while the younger hyphae around the outer edge are thin-walled and hyaline. Those branches of mycelium which come in contact with glass plates usually produce dark-colored, thick-walled chlamydospores or appressoria. Within 2 or 3 days stromata begin to form by abundant branching from a definite point in the mycelium, which finally results in a thick mass of hyphae. These hyphae assume an olivaceous color, and by the fourth day the dark green stromata are macroscopic in size. They form first at the center and later throughout the colony except at the extreme outer edge. Occasionally they are arranged in such a manner as to give the appearance of "fairy rings," but this is not a constant characteristic. The appressoria and the stromata give the young colony an olivaceous appearance. It becomes darker and almost black with age as the stromata become denser and more numerous and finally form an almost homogeneous stromateoid layer at the surface of the substrate.

By the second day the colony shows a small amount of white aerial mycelium. This increases somewhat with age and later takes on a smoky gray appearance, masking the stromateoid layer to a certain extent. In from three to five days fruiting bodies are formed on the stromata at the center of the colony, and they continue to develop as the colony grows. Conidia are produced in abundance in most strains, accumulating in cream-colored or pinkish masses on the fruiting bodies.

The colony will continue to grow to an indefinite size if space and nutrients are available. A diameter of about 25 mm. is reached in seven days at room temperatures.

ON POTATO AGAR (2 PER CENT DEXTROSE) SLANTS.—Growth is similar in most respects to that on plates. Aerial mycelium tends to be more abundant. Mycelium does not, as a rule, extend deeply into the agar to form stromata. As the culture dries out the aerial mycelium forms a

dense mat over the surface of the culture, its color usually becoming slightly brownish with age. Spore masses often appear above this layer of mycelium.

ON OTHER MEDIA.—The growth of the fungus was studied on 25 kinds of artificial media, including beef broth agar, corn meal agar, oat agar, apple agar, synthetic agars, vegetable agars, cooked vegetables, and fresh vegetable tissues. The character of growth on the various media used was so uniform and so closely parallel to that on potato agar that a separate description for each is unnecessary. The most noticeable difference was that correlated with the supply of sugar in the medium. Where dextrose was omitted in the formula growth and sporulation were very scanty, and the stromata were few in number and widely scattered. On onion and apple agars made up without dextrose this difference was less marked, probably on account of the presence of a considerable amount of sugar in the plant tissues used. On synthetic agars¹ with sugar added in the form of maltose, dextrose, lactose, and sucrose copious growth took place with no evidence of preference for any one of the carbohydrates used. Cooked bean pod, onion scale, carrot, potato, and rice supported good development of the organism. On fresh onion and apple, however, the growth was much retarded, and on fresh potato and carrot it was very scanty. Stevens and True (30) report retarded growth on onion broth agar made with red or yellow varieties. The writer has found equally vigorous development on agar made from red, yellow, and white types of onion.

RELATION OF TEMPERATURE TO GROWTH

Potato agar plates inoculated with mycelium or conidia of the fungus were kept at temperatures ranging from 1° to 35° C. The rate of growth was determined by measuring the diameter of the resulting colonies or thalli from day to day. In order to increase the accuracy of the results Petri dishes of equal diameter containing equal amounts of agar were used. In order to overcome the influence of variations in relative humidity prevailing in different incubators the later experiments were modified by placing the Petri dishes in moist chambers first and then exposing them to the desired temperature. It was found after many trials that the best comparative data could be secured at four to six days. The growth was slight at 1°, almost negligible at 2°, but an appreciable amount occurred at 8° to 10° during a period of 10 to 14 days. Above this point the rate of growth increased rapidly, reaching the optimum at about 26°. At 31° to 32° little or no growth occurred on potato agar. The growth at various temperatures on this medium at the end of 6 days is represented graphically in figure 4.

¹ Formula for synthetic agar used: Sugar, 100 gm.; peptone, 20 gm.; ammonium nitrate, 10 gm.; magnesium sulphate, 2.5 gm.; potassium nitrate, 5 gm.; acid potassium phosphate, 2.5 gm.; calcium chloride, 0.1 gm.; agar, 20 gm.; neutralized with normal sodium hydroxid.

A similar study of growth in tubes of onion decoction was made, with essentially parallel results. The optimum on this medium appeared to be slightly higher (27° to 29° C.) and slight growth occurred at 31°.

SPORE GERMINATION

RELATION OF MEDIUM.—For the studies upon spore germination a few drops of the liquid medium to be used were placed in Van Tieghem cells. A suspension of conidia in the same liquid was made, and a drop of this was transferred to cover glasses, which were then inverted over the cells and partially sealed with vaseline. The preparations were placed in Petri dishes and exposed to the desired conditions. For some purposes open drops on glass slides placed in Petri dishes lined with moistened filter paper were more suitable.

A comparative study of spore germination in distilled water, onion decoction,¹ onion leaf extract,² onion scale extract,³ soil extract (sterilized and unsterilized),⁴ and soil decoction⁵ was made.

At room temperature germination in favorable liquid medium began within 5 to 6 hours. At 24 hours practically all viable spores had germinated. The percentage of germination in the drops was determined by averaging the counts of several microscopic fields. The results of these tests are summarized in Table I.

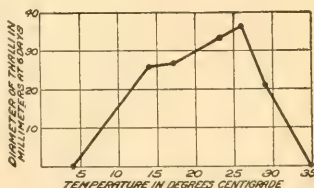


FIG. 4.—Relation of temperature to growth of *Colletotrichum circinans* on agar plates.

TABLE I.—Effect of various media upon spore germination of *Colletotrichum circinans*

Medium.	Percentage of germination.
Distilled water.....	60
Soil decoction.....	95
Soil extract, sterilized.....	95
Soil extract, unsterilized.....	10
Onion decoction.....	99
Onion leaf extract.....	0
Onion leaf extract, diluted with distilled water 1 to 10.....	0
Onion scale extract.....	0
Onion scale extract, diluted with distilled water 1 to 10.....	0

¹ Onion decoction: 100 gm. onion scale in 500 cc. distilled water steamed one hour, filtered, and sterilized.

² Onion leaf extract: Fresh onion leaves (green) crushed and the sap extracted by squeezing through cheesecloth.

³ Onion scale extract: Fresh onion scale crushed and the sap extracted as in onion leaf extract.

⁴ Soil extract: 500 gm. black loam soil was supported in a glass funnel by excelsior and absorbent cotton, 500 cc. of tap water were poured over the soil; the filtrate was collected twice, and each time it was poured over the soil. The third filtrate was divided into two parts; one part was left unsterilized and the other part was sterilized in tubes at 15 pounds pressure for ½ hour.

⁵ Soil decoction: 500 gm. of black loam soil, to which had been added 500 cc. of distilled water, was steamed at 15 pounds pressure for ½ hour. The liquid was filtered through filter paper and sterilized in tubes at 15 pounds pressure for ½ hour.

The striking outcome of this comparison is the marked retardation in unsterilized soil extract and the complete inhibition in onion leaf and onion scale extract. Even when the last two were diluted with 10 parts of water no germination occurred. As pointed out in a previous note by the writer (38), further experiments have shown the presence of at least two distinct substances in onion tissue which are probably responsible for inhibition of spore germination. A more detailed study of this phase and its relation to the parasitism of the fungus will be included in another paper. Cooked soil extract, soil decoction, and onion decoction stimulate germination and promote rapid growth of the germ tubes. It is evident that the cooking of the onion scale removes or destroys the substances which are unfavorable for spore germination.

RELATION OF TEMPERATURE.—Since conidia were found to germinate well in distilled water, this medium was used for studies of the effect of temperature on spore germination. A large number of tests were run at a gradation of temperatures ranging from 1° to 35° C. Spores were

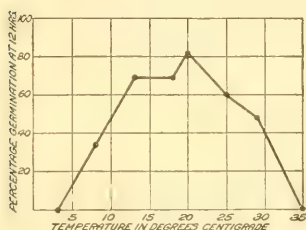


FIG. 5.—Relation of temperature to spore germination of *Colletotrichum circinans*.

found to germinate between the limits of 4° and 32°. Appressoria developed in germination drops throughout the same range of temperature. At 35° to 37° slight swelling of the spores took place, giving them the appearance of "involution forms," but normal germination did not occur. Figure 5 is a graphic representation of the effect of temperature as indicated by percentage of conidia germinating in distilled water at 12 hours. Best germination occurred at about 20°, but good germination occurred between 13° and 25°.

The temperature range for spore germination thus coincides closely with that of fungous growth. The point of optimum development is comparatively high, and this fact is significant in explaining the occurrence of the disease in the field.

EFFECT OF DESICCATION

In order to interpret more fully the development of the disease in the field and the overwintering of the causal organism, the effect of desiccation on conidia and stromata was studied in the laboratory.

ON CONIDIA.—Studies were made on conidia as they occur (1) in masses on the fruiting body on the host, where they are embedded in the mucilaginous material which surrounds them, (2) in similar masses on potato agar, and (3) in water suspension, where the spores are separated from one another, approximating to some extent conditions as

they occur in nature when spores are disseminated by meteoric water. Diseased onions bearing spore masses were brought in and allowed to dry out gradually in the laboratory, and the viability of the spores was tested from time to time. Ordinarily a large percentage lost their vitality within 2 weeks, but in some cases good germination occurred after 7 weeks. A small percentage of conidia from spore masses produced on potato agar and exposed to similar conditions germinated after 4 months. Spores in water suspension allowed to dry out on glass slides were very sensitive to desiccation, little or no germination occurring after 24 hours. It is evident, then, that the conidia are sensitive to desiccation except when they remain in waxy masses on the host, in which condition a small percentage will remain viable through extended unfavorable periods. These results are in accord with the findings of Hasselbring (14) for the somewhat closely related fungus *Gloeosporium fructigenum*, causing the bitter-rot of apple.

ON STROMATA.—The stromata of the fungus are capable of withstanding very long periods of desiccation. Test tube cultures of the fungus on a large number of media were kept at room temperature for a period of two years. Since the tubes were not plugged very tightly with cotton the cultures dried out completely within four or five months. The vitality of the fungus in this desiccated condition was tested by adding sterile melted potato agar to the tube and slanting them until the fresh medium hardened. Vigorous growth characteristic of the fungus resulted from the cultures originally made on potato, beef broth, carrot, corn meal, oatmeal, and onion agars, steamed rice and bean pods, and fresh potato and onion plugs. The fungus was no longer viable on synthetic agar, steamed potato, carrot, onion, and fresh carrot. Since spores lose their vitality in such a long period of drying, it may be inferred that the fungus lived through this extended period of desiccation by means of the stromata which developed in the substrate. It is to be expected from these results that the stromata which develop in the scales of the host are capable of carrying the fungus over long periods of unfavorable climatic conditions.

EFFECT OF FREEZING

ON CONIDIA.—Spores in water suspension exposed to freezing temperatures are killed within a few hours. Fresh spore masses also are very sensitive to low temperatures, but if they are allowed to dry out before being exposed to freezing temperatures they will withstand such temperatures for a month or more. In order to test the resistance of conidia to the freezing weather of the entire winter period, infected onion bulbs bearing spore masses were placed out of doors in a weather instrument shelter at Madison, Wis., on December 7, 1915. Germination tests showed a high percentage of these conidia to be viable at this

time. Tests made on January 22, 1916, showed that by this date all the spores had been killed. A similar experiment was carried out at Madison in the winter of 1919-20. Infected bulbs bearing abundance of spore masses were placed out of doors in October, 1919, and protected from rain and snow. A few viable spores were obtained on March 20, 1920. Thus, a few conidia may withstand Wisconsin winters if sufficiently protected, but probably few, if any, live over under field conditions.

ON STROMATA.—Agar cultures containing abundant stromateoid development were kept out of doors during the winter months at Madison, Wis., during which period there was much severely cold weather. In all cases the cultures were found to be viable at the end of this time. Stromata on onion scales have also been exposed in this region during the winter period, and in every case they withstood the severe freezing temperatures.

It is to be expected from the foregoing data that spore masses withstand short intervals of dry weather during the summer and furnish ready inoculum upon the return of moist conditions. During extended periods of unfavorable conditions, however, the stromata serve best to perpetuate the fungus.

PATHOGENICITY

Inoculation experiments were performed on plants at various stages of growth from young seedlings to mature bulbs.

Sterilized greenhouse loam soil was inoculated by spraying with a water suspension of spores at the time of sowing onion seed. Three hundred seeds of White Globe variety were planted in the inoculated soil and the same number in uninoculated soil. Ten days later, as the cotyledons were coming through the soil, the attack of the fungus became evident by the rapid collapse of the succulent tissue at any point on the young shoot. Acervuli of the fungus were present and continued to develop on the diseased portions of the plants. Fifteen days after sowing, 64 out of 123 plants in the inoculated pot were diseased, whereas all of the 161 plants in the control pot were healthy. This experiment was repeated several times, and in each case where sterilized soil was inoculated a high percentage of the seedlings were killed. When unsterilized greenhouse soil was used the injury was greatly reduced, the competition of other soil organisms evidently greatly limiting the activity of the smudge fungus. Moreover, damping off of this sort due to smudge has never been noted in old onion set fields, other factors, such as low temperature at this early part of the season, probably limiting the activity of the fungus.

Leaves of half-grown plants were sprayed with a spore suspension and kept in a moist chamber for 24 to 48 hours. The fungus developed and fruited on the lower leaves, which had reached a stage of "physiological old age," but this never occurred on vigorously growing leaves.

The disease was produced many times by means of artificial inoculation of healthy mature onion bulbs with suspensions of spores from pure cultures, and the fungus was readily reisolated. A summary of these inoculations is given in Table II. In certain cases when bulbs kept in a closed chamber were thus inoculated, the experiment was unsuccessful. It was found in such instances that although the spores were capable of germination in water, they did not germinate in the drops on the bulbs. The inhibitive effect of the volatile oil of onion on spore germination was mentioned earlier by the writer (38). An accumulation of this substance when several onion bulbs are placed in the small space in a moist chamber may possibly account for this lack of germination. Further studies on this point will be described in a later paper.

More nearly uniform results were secured when sterilized soil was inoculated by spraying with a spore suspension and healthy bulbs then inserted in this medium for a week or 10 days. The outer scales usually became uniformly infected in 7 or 8 days (see Pl. 81, C). When the bulbs were removed and placed in storage, typical invasion of the underlying scales occurred.

TABLE II.—Summary of inoculation and greenhouse experiments on onion bulbs

Type of inoculation.	Inoculation No.	Date of inoculation.	Method of inoculation.	Inoculated.			Controls.	
				Number of onions used.	Percentage infected.	Number of days before first note of disease.	Number of onions used.	Percentage infected
In moist chambers.	1, a	July 24	Spray....	5	100	12	0	0
	1	7	...do....	1	100	6	1	0
	27	Jan. 8	...do....	5	100	5	5	0
	29	20	...do....	5	80	13	5	0
	32	20	...do....	5	0		5	0
	35	July 20	...do....	4	100	5	1	0
	44	Nov. 21	...do....	5	100	6	5	0
	49	Dec. 16	...do....	5	80	18	5	0
	59	Apr. 12	...do....	3	0		0	0
	61	26	...do....	2	100		0	0
In soil.....	3	Aug. 26		10	100		10	0
	23	Dec. 3		5	100	8	5	0
	45	Nov. 30		15	100	8	15	0
	50	Dec. 16		9	100		9	0

In general, then, the fungus assumes the rôle of a weak parasite. Actively growing portions of the plant are not attacked except in young seedlings grown under certain conditions. In the field the fungus is confined to the outer leaves or scales, the cells of which are dead or essentially functionless. As the plant approaches maturity the dry outer scales of the bulb are invaded, but the normal fleshy scales are not affected at this time. A few cases have been noted where the fungus

attacked growing scales which were being parasitized by the smut fungus, *Urocystis cepulae*, but apparently a weakening of the plant is necessary before actual invasion of the growing parts occurs. Following harvest there is a gradual invasion of the dormant cells of the fleshy scales of the bulb as previously described. The progress here is usually slow, but in a moist, warm environment there may be a more rapid invasion, resulting in decay of the resting central bud of the onion set.

RELATION OF THE CAUSAL ORGANISM TO THE HOST TISSUE

METHODS

Onion bulbs from which the thin outer scales had been removed were placed in moist chambers. Inoculum consisting of a suspension of spores from pure culture in sterile distilled water was applied to the uninjured surface of the exposed scales, either in drops by means of a platinum loop or as a spray from an atomizer.

For the study of penetration a razor section was cut tangentially from the surface of the scale directly beneath the infection drop so as



FIG. 6.—*Colletotrichum circinans*: Stage of penetration of epidermal cell of onion scale at 66 hours after inoculation. Camera-lucida sketch. Approximately $\times 430$.

to contain the epidermis with a few layers of the immediately underlying cells. This was examined directly *in toto* in a water mount, the absence of chlorophyll in the host cells making clearing and staining unnecessary. For the study of the relation of the fungus to the host tissue following penetration, pieces of inoculated scale as well as of naturally infected fleshy scales were fixed in Fleming's medium fixative, washed, dehydrated, embedded in paraffin, and sectioned according to standard methods of procedure. In some material a satisfactory differentiation of fungus and host was secured by omitting the bleaching of the microtome sections (commonly done after using a fixative containing osmic acid), which left the mycelium black, and then counterstaining the host cell walls with orange G. In other cases the iron haematoxylin and Delafield's haematoxylin stains gave satisfactory results.

PENETRATION

Under optimum conditions germination occurs within 10 hours and appressoria are formed, either sessile or at the end of short germ tubes. Usually the appressorium is flattened to some extent on the side adja-

cent to the cuticle. The penetration tube is formed from the flattened side of the appressorium and penetrates the cuticle directly (fig. 6, 7). Blackman and Welsford (6) have pointed out that solution of the host cuticle by invading fungi has never been fully demonstrated; they explain the invasion of bean leaf cuticle by *Botrytis cinerea* as mechanical in nature. The mode of penetration in onion smudge was not definitely ascertained, but it seems highly probable that the germ tube from the adhering appressorium might pierce the thin cuticle by means of mechanical pressure.

SUBSEQUENT DEVELOPMENT

The fungus hyphae, after penetration, develop first between the sub-epidermal wall and the cuticle, which is rather elastic in nature and can be raised considerably without being ruptured. Figure 6 illustrates the extent of invading germ tubes at 66 hours after inoculation. The nature of the penetration tube and the subsequent development beneath the cuticle are shown in figure 7. In certain other anthracnose fungi—namely, *Colletotrichum lagenarium* as reported by Gardner (12), *C. lindemuthianum* by Dey (11), and *Gloeosporium fructigenum* by Hasselbring (14)—the penetration tube has been described as invading the cell wall directly. This is also the case in *Botrytis cinerea* on bean (6), although the germ tube in this instance does sometimes grow horizontally beneath the cuticle. The softening of the subcuticular wall in the case of onion smudge soon becomes apparent by its swelling and taking on a laminate appearance. The hyphae grow through and between the laminae (fig. 8) and by rapid development soon form the beginning of the stroma previously described. The swelling of the outer wall eventually involves the entire lumen of the epidermal cell. Although the greatest amount of fungus growth at this stage takes place just beneath the cuticle, occasional hyphae penetrate underlying cells. As the hyphae attack these cell walls, softening and lamination take place as in the subcuticular wall, while penetration is seemingly accomplished partly by means of chemical action and partly by mechanical pressure. The relation of mycelium to the parenchyma cells just beneath the epidermal layer is also shown in figure 8. In the case of bulbs inoculated in moist chambers the collapse of invaded cells was not rapid, and there was no evidence noted of injury to the cells in advance of the mycelium.

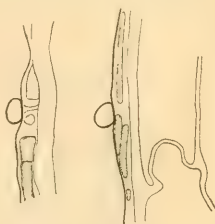


FIG. 7.—Cross section of epidermis, showing early stage of penetration by *Colletotrichum circinans*. Note the empty appressoria with mycelium still wedged between the cuticle and the subcuticular wall. Material fixed 72 hours after inoculation. Camera-lucida sketch. $\times 700$.

Under ordinary storage conditions, the progress of the fungus is closely parallel to that just described, except that the progress is much slower under this different environment. As described before, the first macroscopic symptom of invasion from spots on the dry outer scale to the underlying fleshy scale is a small, yellowish, slightly sunken area. This usually increases in size very slowly in well-ventilated storage. A cross section of one of these spots is illustrated in Plate 83, A, and a detailed drawing from a similar section is shown in figure 9. The fungus develops extensively at first just beneath the cuticle, and the softening and lamination of the subcuticular wall is very slight. As invasion progresses, hyphae penetrate this wall directly, evidently by chemical solution rather than mechanical pressure, since the cavity is slightly larger than the mycelium and there is no sign of bulging of the wall before penetration is achieved. The collapse of cells beneath the epidermal

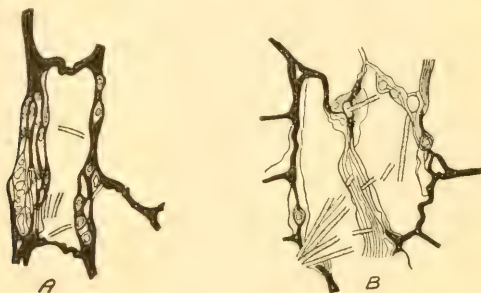


FIG. 8.—Cross section of epidermis (A) and underlying parenchyma cells (B) of onion scale inoculated with a suspension of *Colletotrichum circinans* spores and kept in a moist chamber at room temperature. Note softening and lamination of cell walls by the invading hyphae. Material fixed five days after inoculation. Camera-lucida sketch. A, $\times 308$; B, $\times 350$.

cell takes place before any appreciable invasion of hyphae occurs. In the section shown in Plate 83, A, two layers beneath the epidermal layer have collapsed, while only an occasional hypha is to be found beneath the subcuticular wall. There is no evidence of softening of the cell wall. Moreover, in such lesions mycelium has never been found in the walls or lumina of turgid living cells. This suggests that either the cells are killed in advance of the hyphae or only slight invasion of the wall leads to their collapse. This slow invasion, which prevails even after the cells have become functionless, is surprising in view of what occurs when bulbs are inoculated in moist chambers. Is it possible that the volatile oil present in the onion scale is influential in checking the advance of the fungus?

Under moist conditions and optimum temperature the stroma develops very rapidly in the subcuticular wall, and acervuli are formed in five to

six days after inoculation. This condition is shown in figure 2. In other cases where sporulation is postponed through lack of proper environment the stroma continues its growth more slowly and eventually involves a larger portion of the scale. The cuticle, however, remains intact on the exterior and normally is not ruptured until the palisade layer of conidiophores is formed. A cross section of a scale which had been held in poorly ventilated storage several months is shown in Plate 83, B. Acervuli were produced upon exposure to proper conditions for sporulation. Note that the cuticle is still present outside the extensive stroma, except where it has been ruptured by the sporiferous hyphae.

FACTORS IN THE PRODUCTION AND PROGRESS OF THE DISEASE

OVERWINTERING OF THE CAUSAL ORGANISM

The experiments already reported on the effect of desiccation and freezing upon conidia indicate only a remote possibility that the fungus lives through the winter in this form under Wisconsin conditions. The stromata, on the other hand, are capable of withstanding protracted periods of drouth or freezing temperature. In order to confirm the supposition that the fungus actually overwinters and is widely disseminated in this latter form, four lots of heavily infected bulbs were placed out of doors at Madison, Wis., on December 7, 1915. One lot was left in an instrument shelter near the surface of the ground, and the remaining lots were buried in the soil at depths of 2, 4, and 6 inches, respectively. Spore masses were present on this material at the beginning of the experiment, and germination tests showed a high percentage of the conidia to be viable at this time.

On January 22, 1916, examination of spores from the bulbs placed in the instrument shelter showed that they had completely lost viability by that date. The four lots of bulbs were examined on April 12, 1916. Those which had been buried in soil readily produced conidia in abundance upon exposure to humid conditions at room temperature. The material kept in the instrument shelter had dried out considerably during the winter and, though much slower to respond, eventually proved to be viable by the production of spores. A similar experiment conducted during the winter of 1916-17 yielded confirmatory data.

It is to be expected that infected scales from the crop of the previous season furnish a source of abundant inoculum for initial infection of the growing crop. This, combined with the fact that in most onion-growing sections it is the common practice to grow this crop successively



FIG. 9.—Cross section of onion scale naturally infected with *Colletotrichum circinans*, showing the mycelium developing first just beneath the cuticle and later penetrating the subcuticular wall. Camera-lucida sketch. $\times 450$. (This phase is illustrated further in Pl. 83, A).

on the same field for many years, results in a heavy infection of a large part of the white set crop annually. Examination of a large number of fields in Wisconsin and Illinois has revealed the fact that "clean" white sets are secured as a rule only from land growing its first crop of onions. In a majority of cases the second crop of white sets is badly infected.

In all fields examined where the first crop of onions was being grown, an occasional bulb infected with smudge was found. A satisfactory explanation of these original infections has never been reached. Many possible means of introduction of the fungus from neighboring infected fields immediately suggest themselves, such as manure, farm implements, man and farm animals, drainage water, and wind, and undoubtedly some of these often do play a part in the distribution of the disease. The possibility of seed as a carrier is also to be considered in this connection. Although smudge has never been found attacking the floral parts of the plant, it is conceivable that those seed umbels which fall over and come in contact with the soil before harvest might become infected or be the means of introducing bits of infected scales to the seed. It should be noted in this regard that the spores of onion smut, a disease which is also confined to the bulb and leaves of the plant and in fact does not attack onion seed plants, have previously been found on onion seed samples (9, 18).

One experiment was performed on the relation of seed to the dissemination of the fungus. Samples of six varieties of seed were sown in pots of sterilized soil in the greenhouse on December 5, 1916. On January 16, 1917, all the seedlings were examined. Fruiting bodies of *Colletotrichum circinans* were found on the outer scales of two seedlings of the White Globe variety and of one seedling of the Queen variety. No other signs of the disease were found. The identity of the fungus was confirmed by isolation of pure cultures and comparison with authentic strains. Two subsequent plantings of the same sample of White Globe seed were made, but no further sign of the disease was found. The small amount of the fungus occurring in this experiment is not surprising, since only a very limited amount of infectious material can be expected to be seed-borne. However, although the evidence at hand indicates that the fungus is carried on seed to some extent, further data are necessary before a final conclusion on this point can be made.

RELATION OF TEMPERATURE TO INFECTION AND TO DEVELOPMENT OF THE DISEASE

Studies of the relation of temperature to the germination of conidia and to their subsequent growth have shown the optimum to be about 20° C. for the former and 26° for the latter. The range in each case, however, is wide. Accordingly a set of experiments was started for the purpose of determining the range and optimum temperature for infection.

Sterilized loam soil in glass or glazed crock jars was inoculated with a water suspension of spores. Healthy white onion sets were then

inserted in the soil; and the jars, each covered with a glass plate, were placed in incubators running at temperatures ranging from 5° to 32°.

In the first experiment 10 onions were placed in each of four jars which were placed in incubators held at 5°, 13° to 14°, 23°, and 28° to 31° C., respectively. The extent of the disease on the various lots at this time is shown in Plate 82. It was apparent that infection took place very slowly at 13° to 14°, while that at 28° to 31° was slightly less advanced than at 23°.

In the second experiment jars containing 10 onions each were held at 5° to 6°, 9° to 10°, 14° to 15°, 17° to 18°, 20° to 21.5°, 22° to 23°, 26° to 27°, and 30° to 32° C. They were allowed to remain for 17 days before examination. At the end of this period, no infection had taken place at 5° to 6°, a very slight infection at 9° to 10°, and as the temperature rose the amount of disease increased up to 26° to 27°, at which point it was greater than in any of the other jars. At 31° to 32° it was slightly less than at 26° to 27°. A third experiment confirmed the results of the first two.

Infection takes place and the disease progresses, then, at or above 10° C., but it is quite evident that for very rapid development a temperature of 20° or above is needed. Since the fungus develops in the soil prior to infection, the range of soil temperature during the growing season is undoubtedly an important factor in determining the severity of the disease.

PRODUCTION AND DISSEMINATION OF CONIDIA

After the appearance of the first stromata on the bulbs, subsequent spread of the disease is effected to a considerable extent by conidia.

Sporulation does not take place except under fairly humid conditions. In order to determine the range of temperature at which fructification may occur, infected scales were placed in Petri dishes lined with moistened filter paper and exposed in incubators running at a range of temperatures from 2° to 28° C. Abundant sporulation occurred within 36 hours at 20° to 28°. The process was much retarded at lower temperatures, though a few spores were formed at 2° to 3° after several days.

Under optimum conditions for spore production the conidia accumulate on top of the acervuli, forming gelatinous masses which remain intact among the setae. Exposure of portions of scales bearing fresh spore masses over sterile agar plates has yielded no indication of spore discharge. The mucilaginous material surrounding the spores appears to dissolve partly when a spore mass is placed in water, and the conidia thus become separated.

It is thus to be expected from the nature of the fungus that warm, rainy weather is especially favorable for the development of smudge, since high humidity promotes the production of spores, and meteoric water, especially in the form of spattering rain drops, is important for their dispersion and dissemination.

CORRELATION OF CLIMATIC CONDITIONS WITH THE DEVELOPMENT OF THE DISEASE IN 1915-16

Plots of white onion sets were grown in 1915 and 1916 on land which had previously produced many successive crops of onions and where the smudge organism was known to be present in the soil. Soil temperature records were taken at a depth of 1 to 2 inches during part of the 1915 season and most of the 1916 growing season. The daily mean soil temperatures and rainfall for these seasons are represented in figure

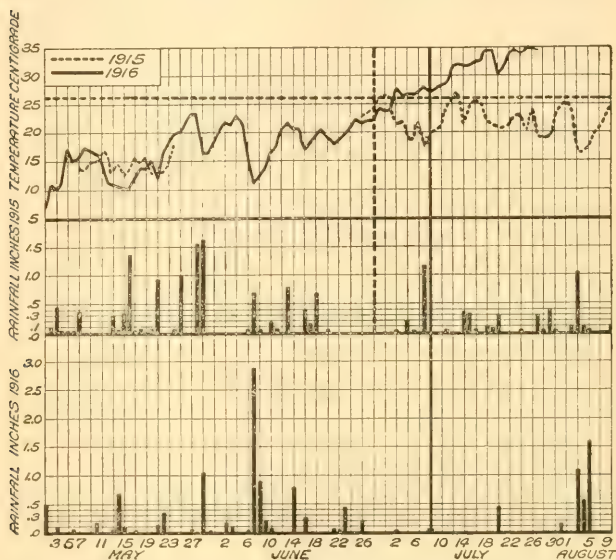


FIG. 10.—Chart from data collected at Racine, Wis., during 1915 and 1916, showing the daily mean soil temperature at a depth of 1 to 2 inches, and the rainfall. The horizontal broken line represents the optimum temperature for infection and development of the disease as indicated by controlled experiments, the broken vertical line the date of first observation of the disease in 1915, and the heavy vertical line the first appearance of the disease in 1916.

10. The rainfall records included here are compiled from data taken at the Racine (Wis.) post office, approximately 3 miles from the onion set plots. The progress of the disease between the time of its first seasonal appearance and harvest is described for these two seasons, since they represent distinctly different conditions which had varying effects upon the progress of the disease.

IN 1915

On June 28 a very few dark green stromata were found, but no acervuli or setae had developed. The soil temperature mean was now well

above 20° C. and remained between 20° and 27° for most of the time until harvest. On July 2 a few scattered acervuli were found. A slight precipitation was recorded on July 2, 2 inches on July 4, 0.02 inch on July 5, and 1.17 inches on July 7. Following this rainy period there was a marked increase in number of acervuli noted on July 10. A slow rain fell during most of July 14 and part of July 15. On July 15 the disease was prevalent above the bulbs on the unthickened portions of the outer leaves which comprise the "neck." These infections were clearly the result of spores scattered upon these portions from the bulb scales by rain a few days previously. The rainy weather, which prevailed until harvest, about August 10, resulted in continued spread and development of the disease, so that the white sets were all badly spotted by the latter date. Further observations showed that the development of the disease in other fields followed closely that noted in the experimental plot. The infection in practically all cases, however, was confined to one or two of the outer dry scales, the fungus being unable to attack the fleshy scales previous to harvest. On the yellow and red varieties the fungus was very abundant on the uncolored portions of the leaves at the neck, but the highly colored bulb scales remained entirely free from it. This has been the usual observation with the colored types.

IN 1916

The month of July, 1916, was extremely warm and dry as contrasted with cool, moist weather of the same period in 1915. The soil temperature mean passed 26° C. on July 2 and remained above that point for the rest of the month. In fact, for a large portion of that period it was well above 32°, the maximum temperature for growth of the fungus on potato agar. No signs of smudge were found until July 8. The extent of the disease at this time was very meager, only a few acervuli being noted. It is probable that the dry weather preceding this date checked the fungus, in spite of the fact that the soil temperature was favorable. Aside from 0.03 inch precipitation on July 8, 0.45 inch on July 20, and 0.14 inch on July 31, no rain fell during the rest of the month. Moreover, the soil temperature was well above the maximum for development of the disease. On July 13 but very little smudge could be found. On July 22 no further development was noted. The moisture from the shower of July 20 disappeared very rapidly from the upper 2 inches of soil because of the extreme heat. A rainy period occurred on August 3, 4, and 5, and following this *Macrosporium porri* and *Phoma alliicola* developed rapidly. Smudge increased but very slowly, however, probably because of the scarcity of viable spores. Another heavy rain fell on August 9 and 10, and the weather then remained clear until after harvest on August 23. At the latter date the bulbs were examined carefully, and in general the sets were only moderately infected. The disease was confined for the most part to the portions of the bulbs below the surface of the soil, while the abundant

infections on the necks which were so conspicuous in 1915 were almost entirely absent.

To summarize, the disease progressed most rapidly during the last part of the growing season of 1915, with the mean temperature range between 20° and 30° C., accompanied by sufficient rainfall to promote abundant spore production and dissemination as well as subsequent infection. On the other hand, development was materially checked in 1916 by extreme heat, together with lack of precipitation during July.

RELATION OF ENVIRONMENT DURING CURING TO THE DISEASE

The onion set crop is usually harvested in early August. The tops are twisted or clipped and the small bulbs are placed in shallow crates 2 or 3 inches deep. These are stacked in the field in piles with temporary roofs, where they are allowed to cure for several weeks. Usually the fungus is well established upon the outer scales of the bulbs before they are pulled, and thus further invasion is dependent largely upon the environmental conditions which prevail during the curing and storage periods.

The respiratory functions of the living cells in the bulbs continue after the sets are pulled, and there is, in consequence, some accumulation of moisture. This is counteracted in part by the use of shallow crates which are exposed to natural air currents. In bright, windy weather the bulbs cure rapidly, while rainy or humid weather retards the process and favors the progress of the disease. A number of experiments were conducted during 1916, 1917, and 1918 to determine the effect of varied amounts of external moisture during the curing period upon the development of the disease.

EXPERIMENT 1.—On August 15, 1916, a crate of white sets was taken from the general run of the crop which had been harvested on August 9 at Racine, Wis. The outer scales were badly spotted with smudge, and in some cases the second scale had been invaded. After removal to the laboratory the bulbs were sprinkled with water while in the crates. After two days a portion of this lot (5½ pounds) was dried for 24 hours at 45° to 52° C. and the remainder (14½ pounds) was given no further treatment. Both lots were placed under cover in a shallow crate, where they were exposed to good conditions for further natural curing. They were later placed in a well-ventilated onion warehouse held at about 35° to 40° F. On January 13, 1917, both lots were examined. Most of the outer dead scales present at harvest time had sloughed off during storage, and in the dried sets the fungus had advanced very little from these original infections. In the naturally cured sets, however, the fungus, probably aided by the greater excess of moisture present, had invaded several underlying scales, and these sets were badly spotted even after the outer scales were removed. The sets in each lot were then sorted into three classes—(1) free from disease, (2) slightly diseased,

(3) badly diseased. The result of this classification is given in Table III, and samples from the dried and the undried lots are shown in Plate 85, A, B.

TABLE III.—*Relation of artificial curing to the development of onion smudge*

Treatment.	Condition at end of storage period.		
	Percentage free from disease.	Percentage slightly diseased.	Percentage badly diseased.
Naturally cured.....	7	29	64
Artificially dried.....	56	36	8

EXPERIMENT 2.—On August 30, 1917, several bushels of white onion sets were secured from a field where the crop had been harvested on August 16 and placed in stacks of shallow crates. The weather had been clear during this intervening period, and good natural conditions for curing had prevailed. Smudge was prevalent on the outer scales of the sets at this time. In order to test the effect of exposure to moist weather on the progress of the disease, a portion of this lot in the crates was sprinkled with water daily for one week, approximating roughly what often occurs when a rainy period comes during harvest. After one week a part of the moistened lot was placed in a kiln drier, where the temperature was held at 100° to 120° F., until the bulbs were thoroughly dried. The remainder of this lot was allowed to dry naturally under cover. All the sets were then stored in a standard onion storage house. Samples taken from a moistened and an unmoistened crate on October 10 are shown in Plate 85, C, D. Marked increase in the amount of smudge was very noticeable within a few days after moistening was begun. On January 14, 1917, the amount of smudge was estimated by classifying several hundred bulbs from each of the three lots into either of two classes, namely, (1) those free from smudge or only slightly diseased and (2) those so badly diseased as to impair their market quality. The results are given in Table IV.

TABLE IV.—*Effect of varied conditions at harvest on the amount of smudge on stored onion sets*

Treatment.	Condition at end of storage period.	
	Percentage free from smudge or slightly diseased.	Percentage badly diseased.
Best natural curing.....	58	42
Exposed to moist conditions after harvest.....	7	93
Artificially dried after exposure to moist conditions.....	52	48

This experiment shows (1) that even under what may be considered very good weather conditions for natural curing a considerable amount of smudge will develop; (2) that exposure to moist weather for a week after harvest practically doubled the amount of smudge; and (3) that thorough artificial drying immediately after such exposure counteracts the effect of excessive moisture.

EXPERIMENT 3.—The sets used in this experiment were from a late sowing and consequently were not harvested until September 14, 1918. Smudge was prevalent on the extreme outer scales of a large percentage of the bulbs at this time. Five bushels were placed in shallow crates in the kiln drier, in which the temperature was maintained at 100° to 120° F. One crate was removed at the end of one day, a second at the end of two days, and the remaining three on the fifth day. Three untreated crates used in the experiment were allowed to cure in a covered pile in the field with the remainder of the crop. On September 30 they were removed to a standard onion warehouse, where they were stored during the winter with the artificially dried lots. On March 5, 1919, when final notes were taken, a comparison of the artificially cured and field-cured lots was secured by estimating the percentage showing any signs of smudge after sets had been milled to remove the loose scales.¹ The results are given in Table V.

TABLE V.—Amount of smudge on artificially cured and field-cured onion sets at the end of the storage period

Crate No.	Nature of treatment.	Length of treatment.	Percentage showing any signs of smudge.
		<i>Days.</i>	
2	Artificially dried.....	1	3
3	do.....	3	22
1	do.....	5	21
4	do.....	5	33
5	do.....	5	31
8	Field-cured.....	16	72
9	do.....	16	75
10	do.....	16	78
	Average of artificially dried crates.....		22
	Average of field-cured crates.....		75

The foregoing experiments clearly establish the importance of moisture as a factor in the advance of the disease during the curing and storage periods. They also indicate that artificial curing immediately following harvest greatly checks the progress of the disease as compared with natural field-curing.

¹ It is the common practice to run "bottom" sets through a fanning mill as they are taken from storage in order to remove the loose outer scales.

RELATION OF STORAGE CONDITIONS TO THE DISEASE

The study of the disease in storage has been directed toward the solution of three problems: (1) The importance of smudge as a cause of premature sprouting of sets; (2) the extent of shrinkage, if any, which can be brought about during the storage of onion sets; and (3) the amount of new infection or actual spread from diseased to healthy bulbs occurring during the holding period. While the data on these points are by no means complete and the factors involved in the progress of the disease during the storage period by no means fully studied, the experiments here reported upon throw some light on the matter.

Observations on the first two questions were made in a standard onion set warehouse at Morton Grove, Ill. In practice, onion sets are stored in crates about 4 inches deep with slatted bottoms, piled so as to allow a 1- to 2-inch space between each two crates to facilitate circulation of air. Sets are placed in storage during September and October. The temperature is gradually lowered, following seasonal changes, until it approaches 0° C. (32° F.), an attempt then being made to hold it slightly above this point. During extremely cold weather some artificial heat in the house is necessary to prevent freezing, while ventilation is constantly needed to remove excessive moisture.

The experiments were carried on during the winter of 1918-19. The extremely mild weather during this season prevented the temperature of the house from being held as close to 0° C. as is commonly the case, while, on the other hand, ample opportunity for ventilation was afforded. Continuous records of temperature and relative humidity were secured by means of a Friez hygro-thermograph. The temperature gradually lowered during October and November, the minimum temperature reaching 0.5° C. (33° F.), on November 23, while the maximum temperature commonly reached 12.7° C. (55° F.) during this period. During December, January, and February the temperature fluctuated between 0.5° and 7.2° C. (33° and 45° F.). The relative humidity varied between 65 per cent and 85 per cent during October and November, while throughout the remainder of the period it seldom went above 75 per cent and not often below 60 per cent.

RELATION OF SMUDGE TO SPROUTING

Two lots of onions were used in these experiments, and, since they differed somewhat as to time of maturity and method of handling, they are here considered separately.

EXPERIMENT 1.—Bulbs averaging about 1 inch in diameter were selected from a lot of white sets harvested early in August and brought into storage on August 22, 1918. Two groups were secured, one consisting of 49 bulbs badly spotted with smudge and the other containing 47 perfectly healthy sets. The two lots had thus been grown and handled alike and presumably differed only as to infection with smudge.

They were carried through storage and examined on February 18, 1919. The results are given in Table VI.

TABLE VI.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 1

Condition of bulbs.	Total number of bulbs used.	Number sprouted.	Percentage sprouted.
Healthy.....	47	14	29. 7
Diseased.....	49	26	53. 0

EXPERIMENT 2.—The sets used in this experiment were sown late in the spring and consequently were not harvested until about September 14, 1918. They were allowed to cure in the field in the normal manner until September 30, when they were placed in storage. Three average crates were selected at this time and kept under observation. At harvest time smudge was prevalent only on the dry outer scales of the sets, but during the storage period it gradually penetrated the underlying scales. When a final examination was made on March 5, 1919, it was clear that in nearly every case where the fungus had penetrated deeply the bulb had sprouted and had thus become worthless. A typical example of this condition is shown in Plate 81, D. An estimate of the amount of sprouting actually due to or intimately associated with smudge was secured by counting 100 to 200 bulbs in each crate. The results are given in Table VII.

TABLE VII.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 2

Crate No.	Number of bulbs examined.	Total percentage infected by smudge.	Total percentage sprouted.	Total percentage sprouted and showing advanced stage of smudge.
1.....	165	75	6. 0	6. 0
2.....	197	75	9. 6	9. 6
3.....	148	72	2. 0	. 7
Average.....		74	5. 8	5. 4

It is not to be construed from these data that smudge is always the chief cause of premature sprouting of onion sets in storage, since unquestionably other factors may often be entirely responsible. One of these, the neckrot decay of the scales, commonly produces a similar effect. It is apparent, however, that the invasion of the bulb scales by the smudge fungus brings about some physiological change which promotes growth of the previously dormant bud.

Economically this factor has considerable value, since bulbs which sprout before the end of the storage period are usually a total loss.

RELATION OF SMUDGE TO SHRINKAGE OF SETS IN STORAGE

In order to secure bulbs as nearly comparable as possible except for presence or absence of smudge, healthy and diseased sets averaging about 1 inch in diameter were selected from a general lot of white sets which had been harvested in early August, properly field-cured, and placed in storage on August 22, 1918. Four lots of 25 bulbs each were secured which showed heavy smudge infection but no signs of any other disease. Three lots of 25 each were selected which appeared to be perfectly healthy. All lots were weighed on October 15. Two diseased lots and one healthy lot were kept in the warehouse throughout the experiment under conditions previously described. In order to secure a high relative humidity a special temporary chamber was made in the warehouse and lined with moistened burlap. Thus, a relative humidity of 90 to 95 per cent was maintained at a temperature close to that of the main warehouse. Two diseased and two healthy lots were placed in this chamber for approximately four weeks and then removed to the main warehouse room. The several lots were weighed on December 30, 1918, and on February 18, 1919. The results of the experiment are given in Table VIII. A constant increase in shrinkage of diseased sets over healthy sets was to be noted. Before the end of the experiment sprouting had occurred in most of the lots, and, as was to be expected, was more prevalent in diseased than in healthy lots. Sprouting and the complication of contaminating parasites should be considered; but, since the former is seemingly enhanced by the disease and the latter is not serious in these cases, there is reason to believe that smudge is responsible in large measure for the increase in shrinkage.

TABLE VIII.—*Relation of smudge to shrinkage of onion sets in storage*

Lot No.	Condition of bulbs.	Environment.	Number of bulbs used.	Original weight, Oct. 15, 1918.	Percentage of shrinkage.		Condition at end of experiment.
					Dec. 30, 1918.	Feb. 18, 1919.	
1	Diseased.....	Ordinary storage.....	25	Gm. 291.8	6.5	18.7	12 sprouting; 1 infected with neck-rot.
18	do.....	do.....	25	277.5	7.4	19.0	15 sprouting.
2	Healthy.....	do.....	25	319.3	2.5	11.3	8 sprouting; 1 infected with blue mold.
3	Diseased.....	Exposure to high relative humidity for 4 weeks, followed by ordinary storage.	25	324.0	8.2	23.3	
20	do.....	do.....	25	303.0	8.9	28.8	16 sprouting; 3 infected with neck-rot.
4	Healthy.....	do.....	25	324.3	2.8	9.1	7 sprouting.
21	do.....	do.....	25	284.5	4.1	11.4	5 sprouting.
Average shrinkage of diseased lots.....					7.7	22.4	
Average shrinkage of healthy lots.....					3.1	10.6	

SPREAD OF SMUDGE IN STORAGE

It has been claimed that smudge spreads from infected to healthy bulbs in storage (17, 29). It is to be expected that under unusually moist conditions this might occur. However, since considerable moisture is necessary for sporulation and infection, the conditions which prevail in good storage houses are not conducive to rapid spread of the disease. Several experiments have been conducted during the course of this investigation in which healthy bulbs have been marked and mixed in lots of badly diseased sets. A summary of these experiments appears in Table IX.

TABLE IX.—*Spread of smudge in storage*

Experiment No.	Storage conditions.	Length of experiment.	Number of healthy bulbs used.	Condition at end of experiment.
		<i>Days.</i>		
1	Standard onion warehouse. . . .	154	34	All healthy.
2	do.....	103	40	2 bulbs showed slight infection.
3	Cool cellar.....	66	20	All healthy.
4	do.....	208	20	Do.
5	Moist chamber at room temperature.	36	20	6 showed slight infection.

It was found that there was little or no spread of the disease under ordinary storage conditions or in a cool cellar. In a saturated atmosphere some infection of healthy bulbs occurred. In practice, then, some spread from diseased to healthy bulbs is to be expected where sets are exposed to rain or very humid atmosphere such as might occur during the curing period. However, with fairly dry sets kept in cool, well-ventilated storage new infections are probably negligible.

CONTROL OF THE DISEASE

The control of this disease is obviously connected closely with the handling of the crop at or immediately following harvest.

In 1915 a spraying experiment was conducted on a plot of white sets at Racine, Wis. The development of the disease in this plot has been described on pages 708-709. Various schedules were used with 4-4-50 and 8-8-50 Bordeaux mixture plus soap, 4-50 copper sulphate, and 1-10, 1-16, and 1-32 lime sulphur. The sprays were applied upon the bulbs and necks of the plants. Contact with the soil probably reduced the disinfecting property of the chemicals, and their adhesiveness was limited by the nature of the scales and leaves of the onion. No beneficial results were secured even where the first application was made before the first signs of the disease appeared and where the spraying was continued at intervals of three to eight days until harvest. The complete failure of

this experiment was sufficient to show that sprays could not be used successfully for the control of smudge.

Dusting of the sets in the crates at harvest time with lime or sulphur has been suggested by Thaxter (33). In 1916 and 1918 dusting experiments with lime, sulphur dust, and dry Bordeaux powder were conducted without any positive results. This is to be expected, since, as a rule, the outer scales of the bulbs became infected before harvest and a disinfectant applied externally could hardly prevent further invasion of underlying scales.

The importance of thorough curing and prevention of exposure to humid conditions after harvest has been emphasized by Thaxter (33), Clinton (10, p. 333), Massee (17), and Stevens and True (30). The experiments reported on the effect of drying of bulbs at harvest have shown that rapid dehydration of the outer scales at this time checks further invasion by the fungus to a large degree. Observations in the field by the writer during the years 1914 to 1920 indicate that even the best natural curing weather to be expected in the Middle West is not sufficient to do more than partially check the disease on seriously infected fields.

Artificial curing offers a possible measure of control for smudge, and, as already pointed out (37), preliminary experiments indicate that neckrot can also be checked by this treatment. Extensive control experiments carried on in the Chicago district in 1918 have shown that thorough drying very soon after harvest is necessary in order to check smudge materially. In the set-growing district a large portion of the crop is grown on contract to be delivered at a central warehouse as soon as it has cured sufficiently. The expense involved in this treatment would almost necessitate that they be dried at a central point, preferably at the place of storage. Therefore, in order to handle the large quantity received, a fairly rapid process of drying would be essential.

Further experimental work is necessary before artificial drying can be recommended as a general practice, and the results of control experiments are reserved for later publication. In the meantime, the most applicable remedial measures consist in prompt harvest and the best use of natural climatic conditions in curing the white onion set crop, including all possible protection from moist weather. This should be followed by storage in a well-ventilated warehouse held as nearly as possible at 33° to 36° F.

SUMMARY

(1) Smudge is one of the most common diseases of white onion sets in Wisconsin and Illinois.

(2) It occurs also on shallot (*Allium ascalonicum*) and leek (*A. porrum*).

(3) The disease was first described by Berkeley in England in 1851 and is now widely distributed in Europe and America.

(4) Smudge is confined to the scales and neck of the bulb, where it causes dark green to black spots. On fleshy scales it appears as sunken yellowish spots which enlarge slowly, coincident with gradual shrinkage of the scale. On colored varieties the disease is confined to unpigmented portions of the outer scales of the neck of the bulb.

(5) Spots on the outer scales of bulbs due to *Macrosporium porri*, *M. parasiticum*, *Phoma alliicola*, and *Urocystis cepulae* may be confused with smudge.

(6) Smudge becomes detrimental to the onion crop as a cause of (1) the reduction of market value of white varieties, (2) shrinkage in storage, and (3) premature sprouting of sets in storage.

(7) A detailed description of the morphology of the causal organism, *Colletotrichum circinans* (Berk.) Voglino, is given. The ascigerous form, *Cleistothecopsis circinans*, has been described by Stevens and True, but complete proof of its connection with *Colletotrichum circinans* is lacking.

(8) Inasmuch as the causal organism produces a subcuticular stroma and a well-defined acervulus, the species is classified in the Melanconiales as *Colletotrichum circinans* (Berk.) Voglino. A comparative study of the latter with *C. fructus* (S. and H.) Sacc. was made.

(9) The characteristic growth of the organism on culture media is described.

(10) Growth on potato agar takes place between 2° and 32° C., while the optimum is about 26°.

(11) Spore germination is stimulated in soil decoction, onion decoction, and sterilized soil extract, as compared with that in distilled water, while it is reduced in unsterilized soil extract and entirely inhibited in onion leaf or scale extract.

(12) Spore germination occurs within the range of 4° and 32° C., while the optimum temperature is from 20° to 26°.

(13) Conidia are very sensitive to desiccation except when in spore masses, in which condition a small percentage retain vitality for four months or more. Stromata are very resistant to desiccation, retaining vitality for two years or more.

(14) Conidia are sensitive to freezing temperatures, but dried spore masses may withstand this environment for a month or more. Stromata are capable of withstanding several months of freezing weather.

(15) The fungus is pathogenic upon the scales of mature bulbs, but does not attack actively growing parts of the plant with the exception of young seedlings, upon which it may cause "damping off" under certain greenhouse conditions.

(16) Spores germinate and appressoria form within 10 to 12 hours. The infection tube is pushed from the side of the appressorium adjacent to the host cuticle directly through the latter. The mycelium then develops for a time between the cuticle and the subcuticular wall, raising

the former and eventually causing a softening of the latter. In bulbs inoculated in moist chambers the fungus progresses fairly rapidly, causing softening and lamination of the walls and the gradual collapse of the cell. The stroma involves the subcuticular wall at first and later the underlying cells, but the cuticle remains unbroken until the acervulus is formed. The process of invasion under storage conditions is essentially the same but much slower.

(17) The fungus overwinters as stromata in infected scales.

(18) Infection occurs at or above 10° C., but progress is very slow below 20°; the optimum is about 26°.

(19) Conidia are produced abundantly under moist conditions and at temperatures between 20° and 30° C. They are disseminated chiefly by meteoric water, especially spattering rain.

(20) The disease develops most rapidly in the field when the mean soil temperature range lies between 20° and 30° C. and is accompanied by abundant rainfall. Extremely hot, dry weather in July checks progress. Presence of moisture favors the progress of the disease during the curing period, whereas artificial drying of sets immediately following harvest checks it.

(21) Smudge tends to promote premature sprouting and increases shrinkage of sets in storage. The disease may spread from bulb to bulb in the crate under very moist conditions, but in proper storage this factor is negligible.

(22) The important measures of control are protection of the harvested crop from rain, rapid and thorough curing, and provision of well-ventilated storage at about 33° to 36° F.

LITERATURE CITED

(1) ALLESCHER, Andreas.

1898-1901. FUNGI IMPERFECTI . . . 1016 p. Leipzig. (Rabenhorst, L. Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2, Bd. 1, Abt. 6.)

(2) ATKINSON, G. F.

1897. SOME FUNGI FROM ALABAMA . . . Bul. Cornell Univ. (Sci.), v. 3, no. 1, 50 p. Bibliography, p. 2.

(3) BENNETT, J. L.

1888. PLANTS OF RHODE ISLAND, BEING AN ENUMERATION OF PLANTS GROWING WITHOUT CULTIVATION IN THE STATE OF RHODE ISLAND. 128 p. Providence, R. I.

(4) BERKELEY, M. J.

1851. [A NEW ONION DISEASE.] *In Gard. Chron.*, 1851, no. 38, p. 595, 2 fig.

(5) ———

1874. NOTICES OF NORTH AMERICAN FUNGI. *In Grevillea*, v. 3, no. 25, p. 1-17. Continued article.

(6) BLACKMAN, V. H., and WELSFORD, E. J.

1916. STUDIES IN THE PHYSIOLOGY OF PARASITISM. II. INFECTION BY BOTRYTIS CINEREA. *In Ann. Bot.*, v. 30, no. 119, p. 389-398, 2 figs., pl. 10. Literature cited, p. 397.

- (7) BRITTON, W. E., and CLINTON, G. P.
[1918.] SPRAY CALENDAR. Conn. Agr. Exp. Sta. Bul. 199, p. 51-98, illus.
- (8) BUBÁK, Fr.
1904. IN BÖHMEN IM JAHRE 1902 AUFGETRETENE PFLANZENKRANKHEITEN.
In Ztschr. Landw. Versuchsw. Österr., Jahrg. 7, Heft 10, p. 731-741.
- (9) CHAPMAN, George H.
1910. NOTES ON THE OCCURRENCE OF FUNGOUS SPORES ON ONION SEED. Mass.
Agr. Exp. Sta. 22d Ann. Rpt., 1909, p. 164-167.
- (10) CLINTON, G. P.
1904. DISEASES OF PLANTS CULTIVATED IN CONNECTICUT. Conn. Agr. Exp.
Sta. 27th Ann. Rpt., 1902/03, p. 279-370, pl. 9-28.
- (11) DEY, P. K.
1919. STUDIES IN THE PHYSIOLOGY OF PARASITISM. V. INFECTION BY COL-
LETOTRICHUM LINDEMUTHIANUM. *In* Ann. Bot., v. 33, no. 131, p.
305-312, pl. 21. References, p. 311.
- (12) GARDNER, M. W.
1918. ANTHRACNOSE OF CUCURBITS. U. S. Dept. Agr. Bul. 727, 68 p., 15 fig.,
8 pl. Literature cited, p. 65-68.
- (13) HALSTED, Byron D.
1891. REPORT OF THE BOTANICAL DEPARTMENT. N. J. Agr. Exp. Sta. 11th
Ann. Rpt., 1890, p. 323-453, illus.
- (14) HASSELBRING, Heinrich.
1906. THE APPRESSORIA OF THE ANTHRACNOSES. *In* Bot. Gaz., v. 42, no. 2,
p. 135-142, 7 fig.
- (15) KEITT, C. W.
1915. SIMPLE TECHNIQUE FOR ISOLATING SINGLE-SPORE STRAINS OF CERTAIN
TYPES OF FUNGI. *In* Phytopathology, v. 5, no. 5, p. 266-269, 1 fig.
- (16) KEMPTON, F. E.
1919. ORIGIN AND DEVELOPMENT OF THE PYCNIDIUM. *In* Bot. Gaz., v. 68, no.
4, p. 233-261, pl. 17-22.
- (17) MASSEE, George.
1903. A TEXT-BOOK OF PLANT DISEASES CAUSED BY CRYPTOGAMIC PARASITES.
ed. 2, 472 p., illus. London, New York.
- (18) MUNN, M. T.
1917. NECK-ROT DISEASE OF ONIONS. New York State Agr. Exp. Sta. Bul.
437, p. 361-455, 11 pl. Bibliography, p. 450-455.
- (19) ORTON, W. A.
1903. PLANT DISEASES IN THE UNITED STATES IN 1902. U. S. Dept. Agr. Year-
book, 1902, p. 714-719.
- (20) ———
1907. PLANT DISEASES IN 1906. U. S. Dept. Agr. Yearbook, 1906, p. 499-508.
- (21) OSNER, George A.
1917. ADDITIONS TO THE LIST OF PLANT DISEASES OF ECONOMIC IMPORTANCE IN
INDIANA. *In* Proc. Ind. Acad. Sci., 1916, p. 327-332.
- (22) PECK, Charles H.
1881. REPORT OF THE BOTANIST. *In* 34th Ann. Rpt. N. Y. State Mus. Nat.
Hist., p. 24-58, 4 pl.
- (23) RUSSELL, H. L.
1915. REPORT OF THE DIRECTOR. PLANT DISEASE SURVEY. Wis. Agr. Exp.
Sta. Bul. 250 (Rpt. 1914), p. 33-39, fig. 14-17.
- (24) SACCARDO, P.
1884-1913. SYLLOGE FUNGORUM . . . v. 3, 1884; v. 4, 1886; v. 22, 1913. Patavii.

- (25) SCHWARZE, Carl A.
1917. THE PARASITIC FUNGI OF NEW JERSEY. N. J. Agr. Exp. Sta. Bul. 313, 226 p., 1056 fig.
- (26) SELBY, A. D.
1910. A BRIEF HANDBOOK OF THE DISEASES OF CULTIVATED PLANTS IN OHIO. Ohio Agr. Exp. Sta. Bul. 214, p. 307-456, 106 fig. List of plant diseases referred to in this publication, p. 1-7.
- (27) ——— and MANNS, T. F.
1909. STUDIES IN DISEASES OF CEREALS AND GRASSES. Ohio Agr. Exp. Sta. Bul. 203, p. 187-236, illus., 14 pl.
- (28) STEVENS, F. L., and HALL, J. G.
1907. AN APPLE ROT DUE TO VOLUTELLA. *In* Jour. Mycol., v. 13, no. 89, p. 94-99, 6 fig.
- (29) ———
1910. DISEASES OF ECONOMIC PLANTS. x, 513 p., illus. New York.
- (30) ——— and TRUE, Esther Y.
1919. BLACK SPOT OF ONION SETS. Ill. Agr. Exp. Sta. Bul. 220, p. 505-532, 19 fig.
- (31) STEWART, F. C.
1900. AN ANTHRACNOSE AND A STEM ROT OF THE CULTIVATED SNAPDRAGON. N. Y. State Agr. Exp. Sta. Bul. 179, p. 105-110, 3 pl.
- (32) STONEMAN, Bertha.
1898. A COMPARATIVE STUDY OF THE DEVELOPMENT OF SOME ANTHRACNOSES. *In* Bot. Gaz., v. 26, no. 2, p. 69-120, pl. 7-18. Bibliography, p. 114-117.
- (33) THAXTER, R.
1890. REPORT OF . . . MYCOLOGIST. *In* Conn. Agr. Exp. Sta. Ann. Rpt., 1889, p. 127-177, 3 pl.
- (34) VAN HOOK, J. M.
1911. INDIANA FUNGI. *In* Proc. Ind. Acad. Sci., 1910, p. 205-212.
- (35) VOGLINO, P.
1907. I FUNGHI PARASSITI DELLE PIANTE OSSERVATI NELLA PROVINCIA DI TORINO E REGIONI VICINE NEL 1906. *In* Ann. R. Accad. Agr. Torino, v. 49, p. 175-202.
- (36) WALKER, J. C.
1917. STUDIES UPON THE ANTHRACNOSE OF THE ONION. (Abstract.) *In* Phytopathology, v. 7, no. 1, p. 59.
- (37) ———
1918. CONTROL OF NECK ROT AND ANTHRACNOSE OF ONION SETS. (Abstract.) *In* Phytopathology, v. 8, no. 2, p. 70.
- (38) ———
1918. NOTES ON THE RESISTANCE OF ONIONS TO ANTHRACNOSE. (Abstract.) *In* Phytopathology, v. 8, no. 2, p. 70-71.

PLATE 8o

Onion smudge:

Onion sets (White Portugal variety) naturally infected with *Colletotrichum circinans*. Collected on August 27, 1919, several weeks after harvest, at Morton Grove, Ill. Photographed September 23, 1919. Note in the three lower bulbs the small sunken spots in the fleshy scales which mark the early stages of invasion of the living tissue. Natural size.

(722)



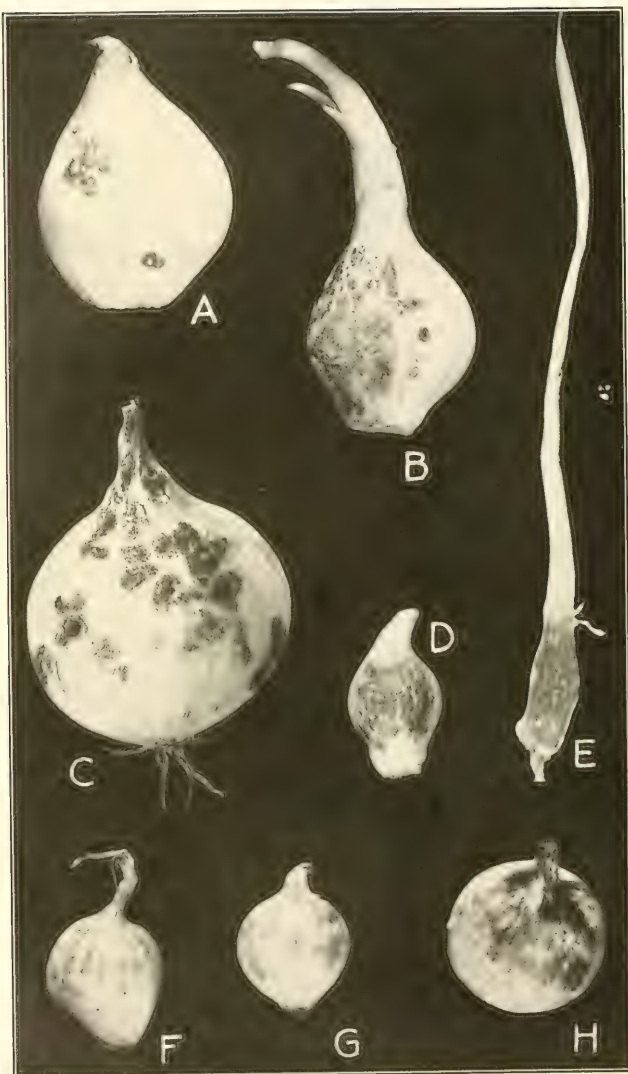


PLATE 81

Onion smudge:

A, B, E, D.—Advanced stages of smudge after several months in storage. Note the shrinkage of fleshy scales and the tendency to sprout.

C.—Bulb inoculated in a moist chamber with a suspension of *Colletotrichum circinans* conidia.

F, G.—*Macrosporium* sp. on outer scale of white onion sets.

H.—*M. porri* and *Phoma alliicola* on outer scale of white onion set. Natural size.

PLATE 82

Relation of soil temperature to the development of smudge:

Onions kept in infected soil held at different temperature for nine days.

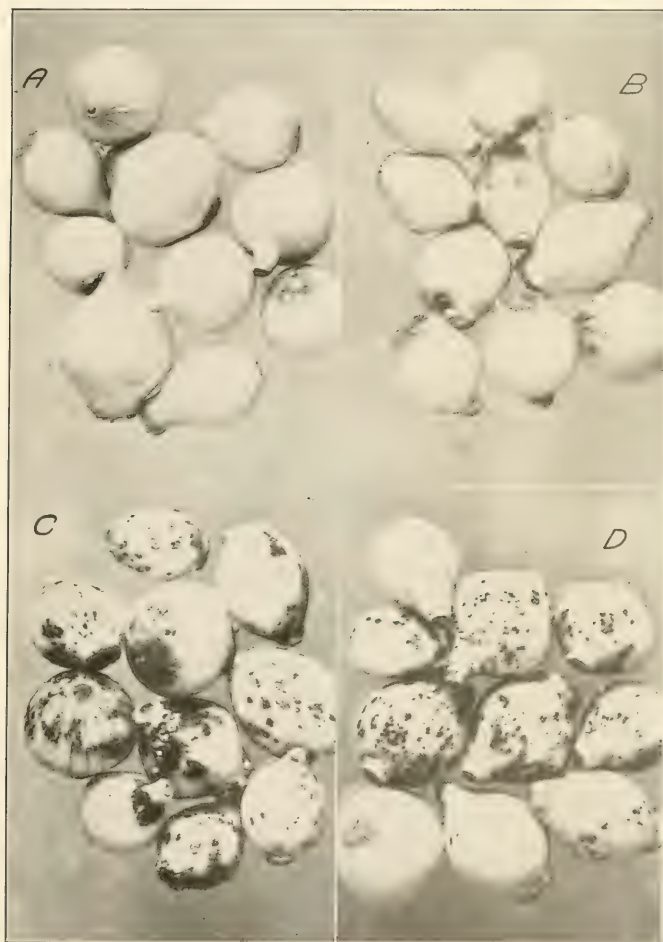
A.—5° C.

B.—15° C.

C.—23° C.

D.—32° C.

Slightly reduced.



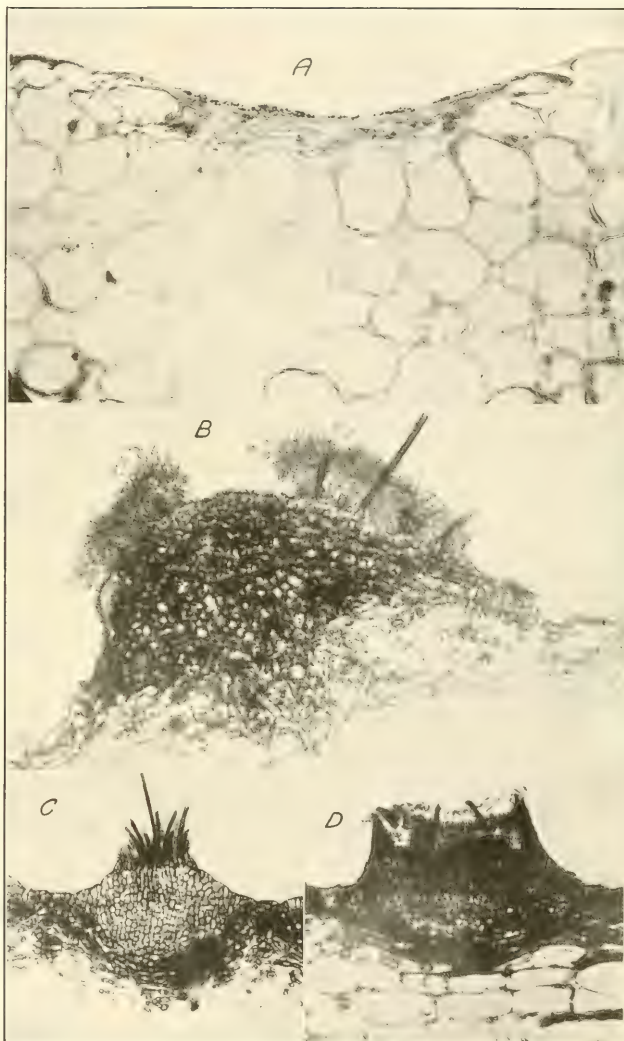


PLATE 83

Colletotrichum circinans and *C. fructus*:

A.—Photomicrograph of cross section of naturally infected onion scale. Note that the fungus is confined largely between the cuticle and the subcuticular wall. The epidermal cells and two layers of the parenchyma cells have collapsed, while the uninvaded cells beneath the lesion are slightly enlarged and distended.

B.—Photomicrograph of cross section of an infected onion scale held for several months in poorly ventilated storage. Note that the stroma is excessively developed and that the cuticle is still intact except where ruptured by the acervuli.

C, D.—Photomicrographs of cross sections of *C. circinans* (C) and *C. fructus* (D) on apple fruit. Note similarity between the two forms and the subcuticular origin of the stromata in each case.

PLATE 84

Colletotrichum fructus and *C. circinans*:

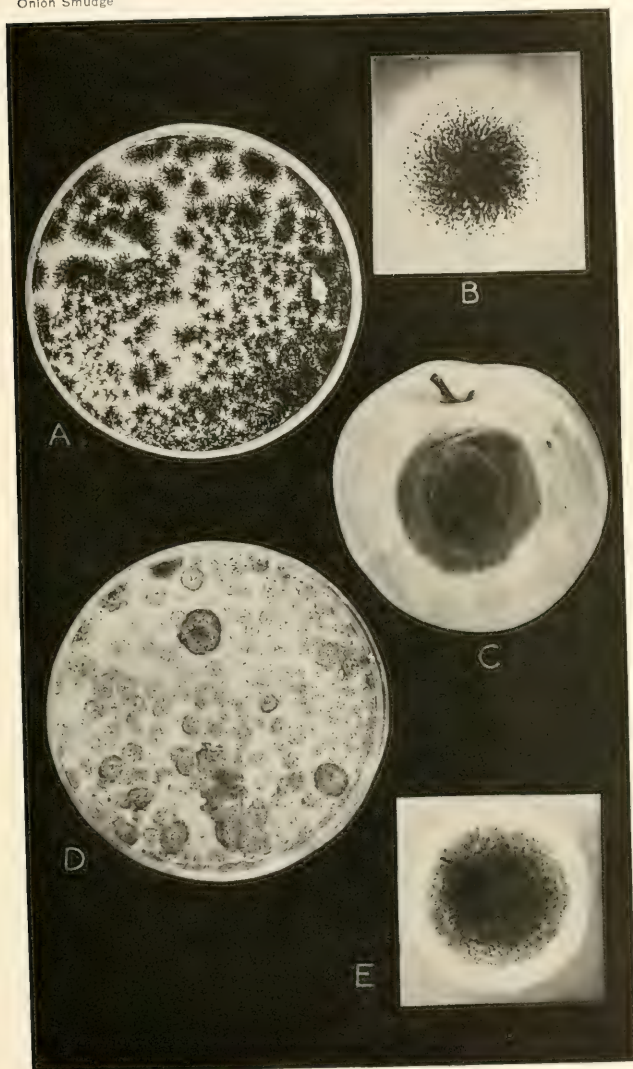
A.—Dilution plate from spores of *Colletotrichum fructus*. Photographed on sixth day. Note stellate character of colonies as compared with *C. circinans* in D. $\times \frac{4}{5}$.

B.—Individual colony of *C. fructus* on potato agar. Photographed on the fourth day. Compare with *C. circinans* in E. $\times 1\frac{3}{4}$.

C.—Apple of Fameuse variety inoculated with mycelium from pure culture of *C. circinans*. Photographed two months after inoculation.

D.—Dilution plate from spores of *C. circinans*. Photographed on sixth day. Compare with *C. fructus* in A. $\times \frac{4}{5}$.

E.—Individual colony of *C. circinans* on potato agar. Photographed on fourth day. Compare with *C. fructus* in B. $\times 1\frac{3}{4}$.



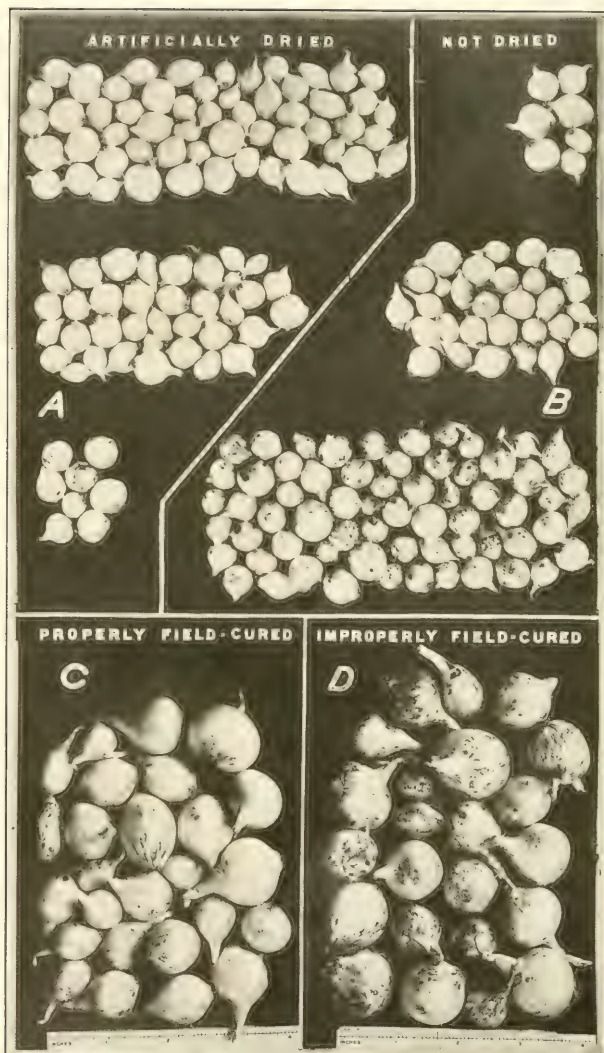


PLATE 85

Relation of curing conditions to the development of smudge:

A, B.—Comparison of onion sets artificially dried immediately after harvest with those not dried. Photograph made at the end of the storage period after the two lots had each been divided into three classes—namely, those free from disease, those slightly diseased, and those badly diseased. (See experiment 1, p. 710-711.)

C, D.—Comparison of white onion sets cured in shallow crates in the field under the best of natural conditions with part of the same lot after exposure to moist conditions for one week. (See experiment 2, p. 711-712.)

LIBRARY OF CONGRESS



0 015 793 352 8